

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
3 July 2003 (03.07.2003)

PCT

(10) International Publication Number
WO 03/053172 A1

- (51) International Patent Classification⁷: **A23L 3/3463**, A23C 19/11, A23B 7/155, 9/28, A23K 1/17
- (21) International Application Number: PCT/EP02/14815
- (22) International Filing Date:
18 December 2002 (18.12.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
01205118.1 21 December 2001 (21.12.2001) EP
- (71) Applicant (for all designated States except US): **DSM N.V.** [NL/NL]; Het Overloon 1, NL-6411 TE HEEERLEN (NL).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **WARMERDAM, Martinus, Johannes, Maria** [NL/NL]; Pauwhof 179, NL-2289 BM RIJSWIJK (ZH) (NL). **RIJN, VAN, Ferdinand, Theodorus, Jozef** [NL/NL]; Valkenlaan 7, NL-2623 HM DELFT (NL). **STARK, Jacobus** [NL/NL]; Joost van Ospad 31, NL-3069 ZM ROTTERDAM (NL).
- (74) Agent: **MATULEWICZ, Emil, Rudolf, Antoni**; DSM N.V., DSM Patents & Trademarks, Office Delft (600-0240), P.O. Box 1, NL-2600 MA DELFT (NL).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/053172 A1

(54) Title: IMPROVED METHOD FOR THE TREATMENT OF FOOD, FEED OR AGRICULTURAL PRODUCTS WITH A POLYENE ANTIFUNGAL COMPOUND

(57) Abstract: The present invention discloses a method for the treatment of a food, feed or agricultural product with a dry powder composition comprising a polyene antifungal compound, the method comprising:- adding the dry powder composition to the food, feed or agricultural product;and- adding an aqueous composition to the food, feed or agricultural product.

IMPROVED METHOD FOR THE TREATMENT OF FOOD, FEED OR AGRICULTURAL PRODUCTS WITH A POLYENE ANTIFUNGAL COMPOUND

5

Field of the invention

The present invention relates to a method for the treatment of food, feed or agricultural products with a dry powder composition comprising a polyene antifungal compound.

The prevention of mould growth is an important topic to the food, feed and agricultural industry, especially in the food industry. Fungal spoilage can lead to serious economic losses. Some foods can be considered a good substrate for fungal growth. Cheese is an example of such a product. Apart from the negative appearance of fungal growth on cheese, fungal spoilage is also a health risk. Some mould species produce mycotoxins, which may penetrate into the product (J.C. Frisvad & U. Thane; "Mycotoxin production by food-borne fungi" in Introduction to food-borne fungi, 4th edn. (ed. R.A. Samson et al.), 1995, 251-260). Therefore, superficial removal of moulds gives no guarantee of safety to the consumer.

For more than 30 years, natamycin has been used to prevent growth of moulds and yeasts on cheeses and sausages. Natamycin is on the market under the brand name of Delvacid®, a powder composition containing 50% (w/w) of natamycin.

Food products can be treated with natamycin in different ways. Natamycin can for example be added to the polymer dispersion that is applied to the cheese rind as a coating (C.B.G. Daamen & G. van den Berg "Prevention of mould growth on cheese by means of natamycin" Voedingsmiddelentechnologie, 1985, 18 (2), 26-29)

Alternatively, food products such as cheeses and sausages can be treated with a suspension of natamycin in water by dipping or spraying (C.B.G. Daamen & G. van den Berg "Prevention of mould growth on cheese by means of natamycin" Voedingsmiddelentechnologie, 1985, 18 (2), 26-29; H.A. Morris & H.B. Castberg "Control of surface growth on blue cheese using pimarin" Cultured Dairy Products Journal, 1980, 15 (2), 21-23; P. Baldini, F. Palmia, R.G. Raczynski, M. Campanini, "Use of pimarin for preventing mould growth on Italian cured meat products", Industria Conserve, 1979, 54

(4), 305-307; R.A. Holley, "Prevention of surface mould growth on Italian dry sausage by natamycin and potassium sorbate", Appl. Environ. Microbiol., 1981, 41 (2).

Spraying of a suspension of natamycin can be applied for the treatment of shredded cheese, a cheese product which is very sensitive to fungal spoilage. The production of shredded cheese is as follows: cheese, e.g. Mozzarella, pizza cheese, or Cheddar is shredded and conveyed to a revolving tumbler. An anti-caking agent, for example cellulose (microcrystalline or powdered), starch or modified starch is metered onto the cheese in the first part of the tumbler. Cellulose forms a film around the cheese particles which prevents caking of the shredded cheese. At the end of the tumbler a suspension of natamycin (e.g. Delvacid®) is sprayed onto the shredded cheese. The spray nozzles deliver a fine spray or mist of the fungicide onto the cheese. Finally, the cheese empties onto a conveyor to be transported to the filling equipment.

The natamycin suspension is prepared by mixing Delvacid® and water, mostly at the ratio from 2 to 10 grams of Delvacid® to 1 litre of water. The suspension is stored in a liquid container. To prevent sedimentation of the suspension a small amount of the suspension, which is pumped under pressure to spray nozzles, is recirculated to the liquid container via a jet agitator. Alternatively, a stirring device can be used to prevent sedimentation. Mostly approximately 6 to 8 litres of Delvacid® suspension is sprayed onto 1000 kg of cheese. Mostly this treatment is effective to prevent fungal spoilage.

Another way to apply natamycin is in the form of a solid dry powder composition containing natamycin and an anti-caking agent, in particular cellulose. Said solid composition may be added to prevent fungal growth on shredded cheese as disclosed in AU 734 084. The solid dry powder composition replaces both the cellulose powder composition and the aqueous natamycin suspension. The amount of natamycin in the solid powder composition may be up to 2% (w/w). Preferably, the amount of natamycin is between 0.02 and 0.5% (w/w). The solid powder composition can be prepared by mixing natamycin with the anti-caking agent, e.g. cellulose, by using for example a tumbler or a convective mixer. The solid powder composition can also be prepared in such a way that a complex between the anti-caking agent and natamycin is formed, e.g. by linking the compounds together. This can be achieved by dissolving both natamycin and cellulose in an aqueous solution by increasing, and subsequent neutralising the pH, which is

followed by drying. All these steps are performed using methods known in the art. The natamycin can also be added at any suitable step in the production process of the anticaking agent.

5 The solid dry powder composition comprising the natamycin can be handled and applied in the same way as the anti-caking agent is applied by methods known in the art. The resulting concentration of natamycin added to shredded cheese as a solid powder composition, lies within the same range as when natamycin has been added as a suspension, which may vary between 2 and 40 ppm of natamycin. The optimal final concentration varies with the type of cheese, the humidity and the production method of
10 the shredded cheese. To prevent fungal growth on other food, feed or agricultural products, the final concentration may be up to 1000 ppm natamycin.

Despite the fact that natamycin may effectively protect food, feed or agricultural products against fungal growth at given concentrations, there is always a need for improvement of the treatment method leading to a more effective protection of said
15 products, which may result in the use of lower concentrations of natamycin.

Summary of the invention

The present invention can provide an improved protection against the development of moulds and yeasts on food, feed or agricultural products.

20 The present invention provides:

(1) A method for the treatment of food, feed or agricultural products with a dry powder composition comprising a polyene antifungal compound, the method comprising:
- adding the dry powder composition to a food, feed or agricultural product; and
- adding an aqueous composition to the food, feed or agricultural product.

25 (2) A method for the treatment of a food, feed or agricultural product, the method comprising adding an aqueous composition to the food, feed or agricultural product which is previously treated with a dry powder composition comprising a polyene antifungal compound.

(3) Use of water to improve the protection against the development of fungal
30 growth on a food, feed, or agricultural product which has been treated by a polyene antifungal compound.

5

Detailed description of the invention

The steps of adding the dry powder composition and the aqueous composition may be performed in any order. Preferably, the dry powder composition is added before the aqueous composition is added. Both compositions may be added by methods known in the art.

10

The amount of the aqueous composition added to the food, feed or agricultural products may be between 0.01% and 5% (v/w), preferably said amount is between 0.5% and 2% (v/w).

15

Any food, feed or agricultural product may be treated with an antifungal compound according to the method of the present invention. Examples of such products are cheese, shredded cheese, grain, animal feed (such as feed for dogs, cats, horses, cows, pigs, poultry and birds), seeds, peanuts and flower bulbs.

20

In a preferred embodiment, the aqueous composition as used in the method according to the present invention is water or is an aqueous solution, an aqueous emulsion or an aqueous dispersion. Preferably, said aqueous composition comprises an antimicrobial compound, for example an antifungal or antibacterial compound.

In another embodiment of the present invention, the antifungal compound in the aqueous composition and/or the dry powder composition is a polyene antifungal compound, preferably said antifungal compound is natamycin.

25

The optimal final concentration of natamycin added according to the method of the present invention, may vary with the type of food, feed or agricultural product. Said concentration may be between 2 and 1000 ppm natamycin, preferably said concentration is between 2 and 500 ppm natamycin, more preferably said concentration is between 2 and 100 ppm natamycin. To prevent fungal growth on shredded cheese, said optimal final concentration may be between 2 and 40 ppm natamycin, preferably said concentration is between 3 and 20 ppm natamycin, more preferably said concentration is between 4 and 10 ppm natamycin.

30

Any ratio wherein natamycin is added, i.e. in a dry powder composition and in an aqueous composition, may be applied. Suitable ratios of natamycin added in a dry powder composition: aqueous composition are in the range from 10% : 90% to 100% : 0%.

- 5 The dry powder composition comprising a polyene antifungal compound may be any known dry powder composition, for example the commercial product Delvacid®, in case the antifungal compound is natamycin.

- 10 The dry powder composition comprising a polyene antifungal compound may also comprise an anti-caking agent. Any anti-caking agent may be used. Examples of suitable anti-caking agents are microcrystalline or powdered cellulose, starch, modified starch, sodium-, magnesium-, potassium-, and zinc silicate, silicium-dioxide, kaolin, talc, potassium and magnesium carbonate, phosphates (di, tri and polyphosphates including sodium, potassium and calcium salts). Also commercial products containing cellulose, such as Keycel or Floam® may be used as anti-caking agent. Further, any combination
15 of anti-caking agents may be used.

- The aqueous composition comprising an antifungal compound, e.g. natamycin, may be prepared by mixing a dry formulation, e.g. Delvacid®, in water using methods known in the art. Preferably a natamycin suspension for spraying is prepared by using a stock suspension of natamycin as described in EP 0 678 241. This stock suspension is a
20 chemically, physically and microbially stable concentrated suspension of natamycin, which provides a convenient stock for the easy and reproducible dosage of a fungicide to food, feed and agricultural products. Apart from natamycin, the suspension comprises a thickening agent, for example xanthan. The concentration of natamycin in the stock suspension may be as high as 40% (w/w), however also stock suspensions with lower
25 concentrations of natamycin can be used. An example of such a stock suspension is the commercial product Delvacid-Sol®. This product comprises 50 to 54% (w/w) of natamycin, 2% (w/w) of xanthan and 44 to 48% (w/w) of lactose. In the case of shredded cheese the stock suspension is diluted with water to the final concentration required for the treatment. This dilution step can be best executed just before adding the natamycin
30 suspension to the shredded cheese.

In case the polyene antifungal compound is natamycin, also complexes of natamycin (e.g. with proteins) and salts of natamycin (e.g. the calcium salt) may be used in the method according to the present invention. Further, the dry and aqueous compositions may also contain components, which are already present in a natamycin preparation. For example, when Delvolid-Instant® or Natamax® is used, lactose will also be present in the final composition.

The following Examples are for illustrative purposes only, and are not to be construed as being limitative to the invention.

Examples

Example 1

Cheddar cheese was shredded using methods known in the art and one of the following formulations of natamycin and cellulose was added to the shredded cheese:

- 15 A) Control, no addition of natamycin;
- B) 2% (w/w) cellulose, followed by spraying with 1% (v/w) of an aqueous natamycin suspension containing 1000 µg/ml (final concentration of natamycin is 10 µg/g of cheese);
- 20 C) 2% (w/w) of cellulose, blended with 500 µg natamycin/g (final concentration of natamycin is 10 µg/g of cheese);
- D) 2% (w/w) of cellulose, blended with 500 µg natamycin/gram, (final concentration of natamycin is 10 µg/g of cheese), followed by spraying with 1% (v/w) of water;
- 25 E) 2% (w/w) of cellulose, blended with 250 µg natamycin/g, followed by 1% (v/w) of an aqueous natamycin suspension containing 500 µg/g (final concentration of natamycin is 10 µg/g of cheese).

After treatment the cheese was stored in plastic bags for 21 days at 4°C. Table 1 shows the presence of molds after 21 days, expressed as log colony forming units per gram of cheese (log cfu/g). The results clearly demonstrate the superiority of a combination of dry and aqueous formulated natamycin over dry or aqueous alone.

Table 1

5

Formulation	Log cfu/g
A: Control	3.4
B: 10 ppm of natamycin added by spraying	2.8
C: 10 ppm natamycin added with cellulose	2.0
D: 10 ppm of natamycin added with cellulose, followed by spraying water 1% (v/w)	1.7
E: 5 ppm of natamycin added with cellulose, followed by 5 ppm of natamycin added by spraying	0.6

Example 2

Cheddar cheese was shredded using methods known in the art and mixed with one of the following formulations of cellulose and natamycin:

- 10 A) Control, no addition of natamycin;
- B) 2% (w/w) cellulose, followed by spraying with 1% (v/w) of an aqueous natamycin suspension containing 500 µg/ml (final concentration of natamycin is 5 µg/g of cheese);
- 15 C) 2% (w/w) of cellulose, blended with 250 µg natamycin/g (final concentration of natamycin is 5 µg/g of cheese);
- D) 2% (w/w) of cellulose, blended with 250 µg natamycin/g (final concentration of natamycin is 5 µg/g of cheese), followed by spraying with 1% (v/w) of water.

20 After mixing with one of the above-mentioned formulations, the shredded cheese was stored in plastic bags for 21 days at 4°C. Table 2 shows the presence of molds after 21 days, expressed as log. colony forming units per gram of cheese (log cfu/g).

The results clearly demonstrate the positive effect of water added after treatment with cellulose blended with natamycin.

5 Table 2

Formulation	Log cfu/g
A: Control	3.4
B: 5 ppm natamycin added by spraying	3.2
C: 5 ppm natamycin added with cellulose	2.4
D: 5 ppm natamycin added with cellulose, followed by spraying water 1% (v/w)	2.1

CLAIMS

1. A method for the treatment of a food, feed or agricultural product with a dry powder composition comprising a polyene antifungal compound, the method comprising:
 - 5 - adding the dry powder composition to the food, feed or agricultural product; and
 - adding an aqueous composition to the food, feed or agricultural product.
2. A method according to claim 1, wherein the dry powder composition is added before the aqueous composition is added.
- 10 3. A method for the treatment of a food, feed or agricultural product, the method comprising adding an aqueous composition to the food, feed or agricultural product which is previously treated with a dry powder composition comprising a polyene antifungal compound.
4. A method according to any one of claims 1 to 3, wherein the aqueous composition comprises an antimicrobial compound.
- 15 5. A method according to claim 4, wherein the antimicrobial compound is an antifungal or antibacterial compound.
6. A method according to claim 5, wherein the antifungal compound is natamycin.
- 20 7. A method according to any one of claims 1 to 6, wherein the aqueous composition is water.
8. A method according to any one of the preceding claims, wherein the aqueous composition is added in an amount between 0.01% and 5% (v/w).
9. A method according to any one of the preceding claims, wherein the polyene antifungal compound in the dry powder composition is natamycin, complexes of natamycin or salts of natamycin.
- 25 10. A method according to any one of claims 6 or 9, wherein the final concentration of natamycin added is between 2 and 1000 ppm.
11. A method according to any one of the preceding claims, wherein the dry powder composition comprises an anticaking agent.
- 30 12. A method according to claim 11, wherein the anticaking agent is cellulose.

13. A method according to claim 11, wherein the anticaking agent is starch or modified starch.

14. A method according to any one of the preceding claims, wherein the product comprises cheese.

5 15. A product treated by a method according to any one of claims 1 to 13.

16. Use of water to improve the protection against the development of fungal growth on a food, feed or agricultural product which has been treated by a polyene antifungal compound.

10

15

20

25

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/14815

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A23L3/3463 A23C19/11 A23B7/155 A23B9/28 A23K1/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23C A23B A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU 734 084 B (GIST BROCADES BV) 31 May 2001 (2001-05-31) cited in the application page 4, paragraphs 1-3; claims 1-6; examples 1-3 page 5, paragraphs 2,3 ---	1,4-15
X	EP 1 068 809 A (PROTEIN TECH INT) 17 January 2001 (2001-01-17) page 4, paragraph 22; claims 6-10,13-16,19-22,24-27,35-37,43-45; examples 1-4 ---	1,4-15
X	WO 97 16195 A (ELI LILLY & COMPANY NZ LIMITED ;MOORE DEREK GEORGE (NZ); LOWE LION) 9 May 1997 (1997-05-09) claims 8,15,16,19 ---	1-15
	-/--	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

21 March 2003

Date of mailing of the international search report

28/03/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Heirbaut, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/14815

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 600 706 A (CARTER A F) 15 July 1986 (1986-07-15)	15
A	the whole document	1-14,16
X	US 5 266 347 A (KING BRUCE D) 30 November 1993 (1993-11-30)	15
A	the whole document	1-14,16
X	US 4 536 494 A (CARTER A FRANKLIN) 20 August 1985 (1985-08-20)	15
A	the whole document	1-14,16
X	KIERMEIER F: "ZUM EISATZ VON PIMARICIN ZUR VERHINDERUNG DER SCHIMMELPILZ- ENTWICKLUNG AUF LEBENSMITTELN ON THE USE OF PIMARICIN FOR GROWTH INHIBITION OF MOULDS IN FOODS" ZEITSCHRIFT FUER LEBENSMITTELN UNTERSUCHUNG UND FORSCHUNG, SPRINGER VERLAG, DE, vol. 151, no. 3, 1973, pages 179-186, XP001025820	15
A	page 183, paragraphs 2-4	1-14,16
X	MORRIS H A ET AL: "CONTROL OF SURFACE GROWTH ON BLUE CHEES EUSING PIMARICIN 1" CULTURED DAIRY PRODUCTS JOURNAL, XX, XX, vol. 15, no. 2, 1 May 1980 (1980-05-01), pages 21-23, XP002062794 cited in the application	15
A	the whole document	1-14,16
X	HOLLEY R A: "PREVENTION OF SURFACE MOLD GROWTH ON ITALIAN DRY SAUSAGE BY NATAMYCIN AND POTASSSIUM SORBATE" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, WASHINGTON,DC, US, vol. 41, no. 2, 1 February 1981 (1981-02-01), pages 422-429, XP002036770 ISSN: 0099-2240 cited in the application	15
A	the whole document	1-14,16

BEST AVAILABLE COPY

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/14815

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
AU 734084	B	31-05-2001	AU 734084 B2	31-05-2001
			AU 7628598 A	28-01-1999
			CA 2243231 A1	18-01-1999
			NZ 331023 A	28-01-1999
EP 1068809	A	17-01-2001	US 6291436 B1	18-09-2001
			AU 4721700 A	18-01-2001
			BR 0000195 A	21-08-2001
			CA 2291382 A1	16-01-2001
			CN 1280774 A	24-01-2001
			EP 1068809 A2	17-01-2001
			JP 2001039805 A	13-02-2001
WO 9716195	A	09-05-1997	NZ 280384 A	29-01-1997
			AU 722767 B2	10-08-2000
			AU 7231996 A	22-05-1997
			BR 9611284 A	26-01-1999
			CA 2236428 A1	09-05-1997
			EP 0858342 A1	19-08-1998
			WO 9716195 A1	09-05-1997
			US 2002031541 A1	14-03-2002
			US 2002051809 A1	02-05-2002
			ZA 9608835 A	27-05-1997
US 4600706	A	15-07-1986	US 4536494 A	20-08-1985
US 5266347	A	30-11-1993	BR 9307906 A	27-08-1996
			WO 9514395 A1	01-06-1995
			AU 5731794 A	13-06-1995
			EP 0730413 A1	11-09-1996
			DE 69329952 D1	29-03-2001
			DE 69329952 T2	09-08-2001
			HK 1013784 A1	23-11-2001
			JP 9511123 T	11-11-1997
			PL 314694 A1	16-09-1996
US 4536494	A	20-08-1985	US 4600706 A	15-07-1986